transition barrier that separates a stalk and the original separate membranes, least energy paths were calculated by the string method. The string method finds a morphological transition between two energy basins_in this case the planar and stalk phases_that minimizes the height of the energy barrier. Sequences of images of bilayer shapes calculated by the string method show that opposing bilayers deform into cusped shapes that then form hydrophobic fissures in each membrane, which then promotes merger into a stalk shape. The energetics of stalk formation and morphologies of stalks were analyzed as a function of lipid composition and bilayer dimensions. The shapes of stalks were compared with shapes of stalks that form between stacks of planar bilayers, as obtained by x-ray diffraction: the theoretically calculated shapes are in agreement with those measured experimentally. Supported by NIH R01 GM101539.

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Variable and Concerted Cooperativity in Snare-Mediated Memebrane Fusion

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The soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex drives the majority of intracellular and exocytic membrane fusion events. If and how SNAREs cooperate to form a single fusion pore has been a subject of intense study with estimates ranging from a single SNARE complex to fifteen being necessary for fusion. Here we show that there is likely no universally conserved number of complexes involved and that this number varies depending on membrane properties. In particular, we found that the threshold for efficient fusion depends on membrane curvature. When docking rates of small (~40 nm) and large (~100 nm) liposomes reconstituted with different synaptobrevin (the SNARE present in synaptic vesicles) densities are taken into account, the fusion efficiency of large SNARE-liposomes declined even when there were more than ~23-30 synaptobrevins present on the entire liposome. The conclusion derived from ensemble measurements that membrane curvature modulates the number of complexes required for fusion was further confirmed by experiments analyzing fusion of single vesicles to planar supported bilayers. We propose that the local number of SNARE complexes required at the site of fusion depends on the energy barrier of a particular fusion reaction and that the complexes assemble in a concerted (non-sequential) fashion for efficient execution of fusion.

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The Snare Motif of Membrane-Anchored Synaptobrevin Exhibits an Aqueous-Interfacial Partitioning that is Modulated by Membrane Curvature

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The SNARE forming motif of the vesicle associated protein synaptobrevin 2, is generally thought to be unstructured in the aqueous phase prior to assembly of the neuronal core SNARE complex. Here, the structure and interfacial association of the full-length vesicle SNARE, synaptobrevin, was compared in four different lipid environments using NMR and EPR spectroscopy. In micelles, segments of the SNARE motif are helical and associated with the interface. However, the fraction of helix and interfacial association decreases as synaptobrevin is moved from micelle to bicelle to bilayer environments, suggesting that the tendency towards interfacial association is sensitive to membrane curvature. In bilayers, the SNARE motif of synaptobrevin transiently associates with the lipid interface, and regions that are helical in micelles are in conformational and environmental exchange in bicelles and bilayers. This work demonstrates that the SNARE motif of synaptobrevin has a significant propensity to form a helix and exchange with the membrane interface prior to SNARE assembly. This transient interfacial association and its sensitivity to membrane curvature and/or defects likely play a role in SNARE recognition events that drive membrane fusion.

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Pre-Fusion Structure of Syntaxin 1A Suggests Pathway for Folding into Neuronal Trans-Snare Complex Fusion Intermediate

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Assembly of the three neuronal SNARE proteins synaptobrevin-2, syntaxin 1A, and SNAP-25 is the key step that leads to exocytotic fusion of synaptic vesicles. In the fully assembled SNARE complex, these three proteins form a coiled-coil four-helix bundle structure by interaction of their respective SNARE motifs. Although biochemical and mutational analyses strongly suggest that the heptad-repeat SNARE motifs zipper into the final structure, little is known about the pre-fusion state of individual membrane-bound SNAREs and how they change conformation from the unzippered pre-fusion to the zippered post-fusion state in a membrane environment. We have solved the solution NMR structure of micelle-bound syntaxin 1A in its pre-fusion conformation. In addition to the transmembrane helix, the SNARE motif consists of two well-ordered, membrane-bound helices separated by the "0-layer" residue Gln226. This unexpected structural order of the N- and C-terminal halves of the uncomplexed SNARE motif suggests the formation of partially zippered SNARE complex intermediates with the "0-layer" serving as a proof-reading site for correct SNARE assembly. Interferometric fluorescence measurements in lipid bilayers confirm that the open SNARE motif helices of syntaxin interact with lipid bilayers and that association with the other target-membrane SNARE SNAP-25 lifts the SNARE motif off the membrane as a critical prerequisite for SNARE complex assembly and membrane fusion.

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Complexin-1 Enhances the On-Rate of Vesicle Docking via Simultaneous Snare and Membrane Interactions

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In synaptic terminals, complexin is thought to have inhibitory and activating roles for spontaneous mini-release and evoked synchronized neurotransmitter release, respectively. We used single vesicle-vesicle microscopy imaging to study the effect of complexin-1 on the docking on-rate between vesicles that mimic synaptic vesicles and vesicles that mimic the plasma membrane. We found that complexin-1 enhances the on-rate of docking between synaptic vesicle mimics containing full-length synaptobrevin-2 and full-length synaptotagmin-1 and plasma membrane mimicking vesicles containing full-length syntaxin-1A and SNAP-25A. This effect requires the C-terminal domain of complexin-1 which binds to the membrane, the presence of PS in the membrane, and the core region of complexin-1 which binds to the SNARE complex.

References

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Quantitative Molecular Modeling of Membrane Curvature Induction by an Amphipathic Helix

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The cell must create membrane curvature in vesicle formation processes like clathrin-mediated endocytosis and SNARE-mediated exocytosis.
Two mechanisms of curvature induction have been studied: Scaffolding, in which a curved protein complex enforces its own shape on the membrane, and hydrophobic insertion, in which peptide material inserts directly into the bilayer and modifies the surface properties.
This work studies the latter.
A detailed molecular model (the CHARMM forcefield) of an amphipathic helix embedded at the surface of the lipid membrane is shown to create a substantial curvature preference.
The model is directly contrasted with the prediction of weaker induction by a continuum elastic treatment.
The discrepancy is analyzed in terms of how curvature is influenced by lipid/inclusion shape and specific chemical interactions.